Please delete the paragraph on page 5, line 4, beginning with "Figure 7" and replace it with the following rewritten paragraph:

B1

Figure 7 depicts a nucleotide and amino acid sequence (SEQ ID NOS: 3 and 4, respectively) of a CTLA4 mutant molecule ("L104EA29YIg") comprising a signal peptide; a mutated extracellular domain of CTLA4 starting at methionine at position +1 and ending at aspartic acid at position +124, or starting at alanine at position -1 and ending at aspartic acid at position +124; and an Ig region as described in Example 1, *infra*.

Please delete the paragraph on page 5, line 9, beginning with "Figure 8" and replace it with the following rewritten paragraph:

B2

Figure 8 depicts a nucleotide and amino acid sequence (SEQ ID NOS: 5 and 6, respectively) of a CTLA4 mutant molecule ("L104EIg") comprising a signal peptide; a mutated extracellular domain of CTLA4 starting at methionine at position +1 and ending at aspartic acid at position +124, or starting at alanine at position -1 and ending at aspartic acid at position +124; and an Ig region as described in Example 1, *infra*.

Please delete the paragraph on page 5, line 14, beginning with "Figure 9" and replace it with the following rewritten paragraph:

B3

Figure 9 depicts a nucleotide and amino acid sequence (SEQ ID NOS: 7 and 8, respectively) of a CTLA4Ig having a signal peptide; a wild type amino acid sequence of the extracellular domain of CTLA4 starting at methionine at position +1 to aspartic acid at position +124, or starting at alanine at position -1 to aspartic acid at position +124; and an Ig region.

Please delete the paragraph on page 6, line 23, beginning with "'CTLA4Ig' is" and replace it with the following rewritten paragraph:

B4

"CTLA4Ig" is a soluble fusion protein comprising an extracellular domain of wild type CTLA4, or a portion thereof that binds CD80 and/or CD86, joined to an Ig tail. A particular embodiment comprises the extracellular domain of wild type CTLA4 starting at methionine at position +1 and ending at aspartic acid at position +124; or starting at alanine at position -1 and ending at aspartic acid at position +124; a junction amino acid residue glutamine at position +125; and an immunoglobulin portion encompassing glutamic acid at position +126 through lysine at position +357 (Figure 9; SEQ ID NO: 8).

Please delete the paragraph on page 9, line 1, beginning with "As used herein 'the extracellular domain of CTLA4'" and replace it with the following rewritten paragraph:

B5

As used herein "the extracellular domain of CTLA4" is a portion of the CTLA4 that recognizes and binds CD80 and/or CD86. For example, an extracellular domain of CTLA4 comprises methionine at position +1 to aspartic acid at position +124 (Figure 9; SEQ ID NO: 8). Alternatively, an extracellular domain of CTLA4 comprises alanine at position -1 to aspartic acid at position +124 (Figure 9; SEQ ID NO: 8). The extracellular domain includes fragments or derivatives of CTLA4 that bind CD80 and/or CD86.

Please delete the two consecutive paragraphs on page 10, lines 1 and 13, beginning with "Examples of" and "CTLA4 mutant molecules" and replace them, with the following rewritten paragraphs:

B6

Examples of CTLA4 mutant molecules include L104EA29YIg (Figure 7; SEQ ID NOS: 3 and 4). The amino acid sequence of L104EA29YIg can begin at alanine at amino acid

position -1 and end at lysine at amino acid position +357. Alternatively, the amino acid sequence of L104EA29YIg can begin at methionine at amino acid position +1 and end at lysine at amino acid position +357. The CTLA4 portion of L104EA29YIg encompasses methionine at position +1 through aspartic acid at position +124. L104EA29YIg comprises a junction amino acid residue glutamine at position +125 and an immunoglobulin portion encompassing glutamic acid at position +126 through lysine at position +357 (Figure 7; SEQ ID NO: 4) L104EA29YIg binds approximately 2-fold more avidly than wild type CTLA4Ig (hereinafter referred to as CTLA4Ig) to CD80 and approximately 4-fold more avidly to CD86. This stronger binding results in L104EA29YIg being more effective than CTLA4Ig at blocking immune responses.

CTLA4 mutant molecules comprise at least the extracellular domain of CTLA4, or portions thereof that bind CD80 and/or CD86. The extracellular portion of a CTLA4 mutant molecule comprises an amino acid sequence starting with methionine at position +1 through aspartic acid at position +124 (Figure 7 (SEQ ID NOS: 3 and 4) or Figure 8 (SEQ ID NOS: 5 and 6)). Alternatively, the extracellular portion of the CTLA4 can comprise an amino acid sequence starting with alanine at position -1 through aspartic acid at position +124 (Figure 7 (SEQ ID NOS: 3 and 4) or Figure 8 (SEQ ID NOS: 5 and 6))

Please delete the paragraph starting on page 10, line 29, beginning with "In another embodiment" and replace it, with the following rewritten paragraph:

In another embodiment, the soluble CTLA4 mutant molecule is a fusion protein comprising the extracellular domain of CTLA4 having one or more mutation in or near a region of an amino acid sequence beginning with methionine at +97 and ending with glycine at +107 (M97-G107). For example, leucine at position +104 of wild type CTLA4 can be substituted with glutamic acid (codons: GAA, GAG). A CTLA4 mutant molecule

having this substitution is referred to herein as L104EIg (Figure 8; SEQ ID NOS: 5 and 6).

Please delete the two consecutive paragraphs starting on page 11, lines 5 and 15, beginning with "In yet another embodiment" and "The invention further" and replace them, with the following rewritten paragraphs:

In yet another embodiment, the soluble CTLA4 mutant molecule is a fusion protein comprising the extracellular domain of CTLA4 having one or more mutations in the S25-R33 and M97-G107 regions. For example, in one embodiment, a CTLA4 mutant molecule comprises tyrosine at position +29 instead of alanine; and glutamic acid at position +104 instead of leucine. A CTLA4 mutant molecule having these substitutions is referred to herein as L104EA29YIg (Figure 7; SEQ ID NO: 3 and 4). The nucleic acid molecule that encodes L104EA29YIg is contained in pD16 L104EA29YIg and was deposited on June 19, 2000 with the American Type Culture Collection (ATCC), 10801 University Blvd., Manasas, VA 20110-2209 (ATCC No. PTA-2104). The pD16 L104EA29YIg vector is a derivative of the pcDNA3 vector (INVITROGEN).

The invention further provides a soluble CTLA4 mutant molecule comprising and extracellular domain of a CTLA4 mutant as shown in Figure 7 (SEQ ID NOS: 3 and 4) or Figure 8 (SEQ ID NOS: 5 and 6), or portion(s) thereof, and a moiety that alters the solubility, affinity and/or valency of the CTLA4 mutant molecule.

Please delete the paragraph starting on page 12, line 7, beginning with "The invention further" and replace it, with the following rewritten paragraph:

B9 The invention further provides soluble mutant CTLA4Ig fusion proteins preferentially more reactive with the CD80 and/or CD86 antigen compared to wild type CTLA4. One example is L104EA29YIg as shown in Figure 7 (SEQ ID NOS: 3 and 4).

Please delete the paragraph starting on page 12, line 14, beginning with "In another embodiment" and replace it, with the following rewritten paragraph:

B10 In another embodiment, the soluble CTLA4 mutant molecule includes the immunoglobulin portion (e.g., hinge, CH2 and CH3 domains), where any or all of the cysteine residues, within the hinge domain of the immunoglobulin portion are substituted with serine, for example, the cysteines at positions +130, +136, or +139 (Figure 7 (SEQ ID NOS: 3 and 4) or Figure 8 (SEQ ID NOS: 5 and 6)). The mutant molecule may also include the proline at position +148 substituted with a serine, as shown in Figure 7 (SEQ ID NO: 4) or 8 (SEQ ID NO: 6).

Please delete the consecutive paragraphs on page 13, lines 7 and 22, beginning with "The soluble CTLA4" and "A preferred embodiment" and replace them with the following rewritten paragraphs:

B11 The soluble CTLA4 mutant molecules of the invention can be obtained by molecular or chemical synthesis methods. The molecular methods may include the following steps: introducing a suitable host cell with a nucleic acid molecule that expresses and encodes the soluble CTLA4 mutant molecule; culturing the host cell so introduced under conditions that permit the host cell to express the mutant molecules; and isolating the expressed mutant molecules. The signal peptide portion of the mutant molecule permits the protein molecules to be expressed on the cell surface and to be secreted by the host cell. The translated mutant molecules can undergo post-translational modification, involving cleavage of the signal peptide to produce a mature protein having the CTLA4

and the immunoglobulin portions. The cleavage may occur after the alanine at position -1, resulting in a mature mutant molecule having methionine at position +1 as the first amino acid (Figure 7 (SEQ ID NO: 4) or 8 (SEQ ID NO: 6)). Alternatively, the cleavage may occur after the methionine at position -2, resulting in a mature mutant molecule having alanine at position -1 as the first amino acid.

A preferred embodiment is a soluble CTLA4 mutant molecule having the extracellular domain of human CTLA4 linked to all or a portion of a human immunoglobulin molecule (e.g., hinge, CH2 and CH3). This preferred molecule includes the CTLA4 portion of the soluble molecule encompassing an amino acid sequence having methionine at position +1 through aspartic acid at position +124, a junction amino acid residue glutamine at position +125, and the immunoglobulin portion encompassing glutamic acid at position +126 through lysine at position +357. The portion having the extracellular domain of CTLA4 is mutated so that alanine at position +29 is substituted with tyrosine and leucine at position +104 is substituted with glutamic acid. The immunoglobulin portion of the mutant molecule can be mutated, so that the cysteines at positions +130, +136, and +139 are substituted with serine, and the proline at position +148 is substituted with serine. This mutant molecule is designated herein as L104EA29YIg (Figure 7 (SEQ ID NOS: 3 and 4)).

Please delete the three consecutive paragraphs on page 14, lines 3, 14 and 27, beginning with "Another embodiment", "Another mutant molecule" and "Alternatively," and replace them with the following rewritten paragraphs:

Another embodiment of L104EA29YIg is a mutant molecule having an amino acid sequence having alanine at position -1 through aspartic acid at position +124, a junction amino acid residue glutamine at position +125, and the immunoglobulin portion encompassing glutamic acid at position +126 (e.g., +126 through lysine at position +357).

The portion having the extracellular domain of CTLA4 is mutated so that alanine at position +29 is replaced with tyrosine; and leucine at position +104 is replaced with glutamic acid. The immunoglobulin portion of the mutant molecule is mutated so that the cysteines at positions +130, +136, and +139 are replaced with serine, and the proline at position +148 is replaced with serine. This mutant molecule is designated herein as L104EA29YIg (Figure 7 (SEQ ID NOS: 3 and 4)). After the signal sequence has been cleaved, L104EA29YIg can either begin with a methionine at position +1, or begin with alanine at position -1.

B12 Another mutant molecule of the invention is a soluble CTLA4 mutant molecule having the extracellular domain of human CTLA4 linked to the human immunoglobulin molecule (e.g., hinge, CH2 and CH3). This molecule includes the portion of the amino acid sequence encoding CTLA4 starting with methionine at position +1 through aspartic acid at position +124, a junction amino acid residue glutamine at position +125, and the immunoglobulin portion encompassing an amino acid sequence having glutamic acid at position +126 through lysine at position +357. The portion having the extracellular domain of CTLA4 is mutated so that leucine at position +104 is substituted with glutamic acid. The hinge portion of the mutant molecule is mutated so that the cysteines at positions +130, +136, and +139 are substituted with serine, and the proline at position +148 is substituted with serine. This mutant molecule is designated herein as L104EIg (Figure 8 (SEQ ID NOS: 5 and 6)).

Alternatively, an embodiment of L104EIg is a soluble CTLA4 mutant molecule having an extracellular domain of human CTLA4 linked to a human immunoglobulin molecule (e.g., hinge, CH2 and CH3). This preferred molecule includes the CTLA4 portion encompassing an amino acid sequence beginning with alanine at position -1 through aspartic acid at position +124, a junction amino acid residue glutamine at position +125, and the immunoglobulin portion encompassing glutamic acid at position +126 through lysine at position +357. The portion having the extracellular domain of CTLA4 is

mutated so that leucine at position +104 is substituted with glutamic acid. The hinge portion of the mutant molecule is mutated so that the cysteines at positions +130, +136, and +139 are substituted with serine, and the proline at position +148 is substituted with serine. This mutant molecule is designated herein as L104EIg (Figure 8 (SEQ ID NOS: 5 and 6)).

Please delete the paragraph on page 15, line 8, beginning "Further," and replace it with the following rewritten paragraph:

Further, the invention provides a soluble CTLA4 mutant molecule having: (a) a first amino acid sequence of a membrane glycoprotein, e.g., CD28, CD86, CD80, CD40, and gp39, which blocks T cell proliferation, fused to a second amino acid sequence; (b) the second amino acid sequence being a fragment of the extracellular domain of mutant CTLA4 which blocks T cell proliferation, such as, for example an amino acid molecule comprising methionine at position +1 through aspartic acid at position +124 (Figure 7 (SEQ ID NO: 4) or 8 (SEQ ID NO: 6)); and (c) a third amino acid sequence which acts as an identification tag or enhances solubility of the molecule. For example, the third amino acid sequence can consist essentially of amino acid residues of the hinge, CH2 and CH3 regions of a non-immunogenic immunoglobulin molecule. Examples of suitable immunoglobulin molecules include, but are not limited to, human or monkey immunoglobulin, e.g., C(gamma)1. Other isotypes are also possible.

Please delete the paragraph on page 16, line 1, beginning "The invention" and replace it with the following rewritten paragraph:

The invention includes pharmaceutical compositions for use in the treatment of immune system diseases comprising pharmaceutically effective amounts of soluble CTLA4 mutant molecules. In certain embodiments, the immune system diseases are

mediated by CD28- and/or CTLA4-positive cell interactions with CD80 and/or CD86 positive cells. The soluble CTLA4 mutant molecules are preferably CTLA4 molecules having one or more mutations in the extracellular domain of CTLA4. The pharmaceutical composition can include soluble CTLA4 mutant protein molecules and/or nucleic acid molecules, and/or vectors encoding the molecules. In preferred embodiments, the soluble CTLA4 mutant molecules have the amino acid sequence of the extracellular domain of CTLA4 as shown in either Figures 7 (SEQ ID NOS: 3 and 4) or 8 (SEQ ID NOS: 5 and 6) (L104EA29Y or L104E, respectively). Even more preferably, the soluble CTLA4 mutant molecule is L104EA29YIg as disclosed herein. The compositions may additionally include other therapeutic agents, including, but not limited to, drug toxins, enzymes, antibodies, or conjugates.

Please delete the paragraph on page 18, line 7, beginning "Additionally," and replace it with the following rewritten paragraph:

Additionally, the invention provides methods for regulating functional CTLA4- and CD28- positive T cell interactions with CD80- and/or CD86-positive cells. The methods comprise contacting the CD80- and/or CD86-positive cells with a soluble CTLA4 mutant molecule of the invention so as to form mutant CTLA4/CD80 and/or mutant CTLA4/CD86 complexes, the complexes interfering with reaction of endogenous CTLA4 antigen with CD80 and/or CD86, and/or the complexes interfering with reaction of endogenous CD28 antigen with CD80 and/or CD86. In one embodiment of the invention, the soluble CTLA4 mutant molecule is a fusion protein that contains at least a portion of the extracellular domain of mutant CTLA4. In another embodiment, the soluble CTLA4 mutant molecule comprises: a first amino acid sequence including the extracellular domain of CTLA4 from the amino acid sequence having methionine at position +1 to aspartic acid at position +124, including at least one mutation; and a second amino acid sequence including the hinge, CH2, and CH3 regions of the human

immunoglobulin gamma 1 molecule (Figure 7 (SEQ ID NO: 4) or Figure 8 (SEQ ID NO: 6)).

Please delete the paragraph on page 29, line 15, beginning "A mutagenesis" and replace it with the following rewritten paragraph:

A mutagenesis and screening strategy was developed to identify mutant CTLA4Ig molecules that had slower rates of dissociation ("off" rates) from CD80 and/or CD86 molecules. Single-site mutant nucleotide sequences were generated using CTLA4Ig (U.S. Patent Nos: 5,844,095; 5,851,795; and 5,885,796; ATCC Accession No. 68629) as a template. Mutagenic oligonucleotide PCR primers were designed for random mutagenesis of a specific cDNA codon by allowing any base at positions 1 and 2 of the codon, but only guanine or thymine at position 3 (XXG/T; also known as NNG/T). In this manner, a specific codon encoding an amino acid could be randomly mutated to code for each of the 20 amino acids. In that regard, XXG/T mutagenesis yields 32 potential codons encoding each of the 20 amino acids. PCR products encoding mutations in close proximity to -M97-G107 of CTLA4Ig (see Figure 7 (SEQ ID NOS: 3 and 4) or 8 (SEQ ID NOS: 5 and 6)), were digested with SacI/XbaI and subcloned into similarly cut CTLA4Ig πLN (also known as piLN) expression vector. This method was used to generate the single-site CTLA4 mutant molecule L104EIg (Figure 8 (SEQ ID NOS: 5 and 6)).

Please delete the paragraph on page 34, line 2, beginning "Single chain" and replace it with the following rewritten paragraph:

Single chain $CTLA4X_{C120S}$ was prepared as previously described (Linsley et al., (1995) J. Biol. Chem., 270:15417-15424). Briefly, an oncostatin M CTLA4 (OMCTLA4) expression plasmid was used as a template, the forward primer,